ULTRASTRUCTURE OF THE HAEMOCYTES ASSOCIATED WITH THE DORSAL TEGUMENT OF *APIS DORSATA* (HYMENOPTERA, APIDAE) WORKERS [1]

Carminda da Cruz-Landim[2]

ABSTRACT

The presence of haemocytes underneath the enlarged epithelium of III, IV and V tergites of forager workers of *Apis dorsata* Fabricius, 1793, is reported. This epithelium shows the morphology of wax glands in a post-secretory (involutive) phase. The haemocytes are plasmatocytes and granulocytes showing morphological evidence of both phagocytic activity and intracellular digestion. The glandular epithelium, however, remains entire though not actively secreting and effective phagocytosis was not observed.

KEYWORDS. Haemocytes, glandular epithelium, tergite, *Apis dorsata*.

INTRODUCTION

Insects present several different types of haemocytes varying in form and number according to species, physiological state, and body location (GILLIAM & SHIMANUKI, 1971; CRUZ-LANDIM & S.-DA-CUNHA, 1971, GUPTA, 1979). These cells perform several functions, acting during insect growth and differentiation (WHITTEN, 1969); in defense against foreign or noxious agents (FISHER, 1970; FEIR, 1979; RATCLIFF & ROWLEY, 1979; WAGO, 1982; BIDOCHKA & KHACHATOURIAN, 1987); in connective tissue formation (ASHURST, 1979) in epidermis regeneration (LAI-FOOK, 1970).

The social bees present many exocrine glands derived from the tegumentary epidermis, whose occurrence is specifically related to the caste. Development and

1. Supported by FAPESP (Proc. 93/2389-7; 92/4700-0) and CNPq (520987/93-4).

2. Departamento de Biologia e Centro de Estudos de Insetos Sociais, Instituto de Biociências. Universidade Estadual Paulista, 13506-900, Rio Claro, SP, Brazil, FAX (0195) 34-0009, E-mail UERCB@BRFAPESP.BITNET.

secretory cycles of these glands are characteristically linked to the individual's role in the society (MOTA & CRUZ-LANDIM, 1988; CRUZ-LANDIM & MOTA, 1993).

The haemocytes close to the epidermis in foragers workers is described and discussed the association, morphology and possible relationships of glandular structures and blood cells.

MATERIAL AND METHODS

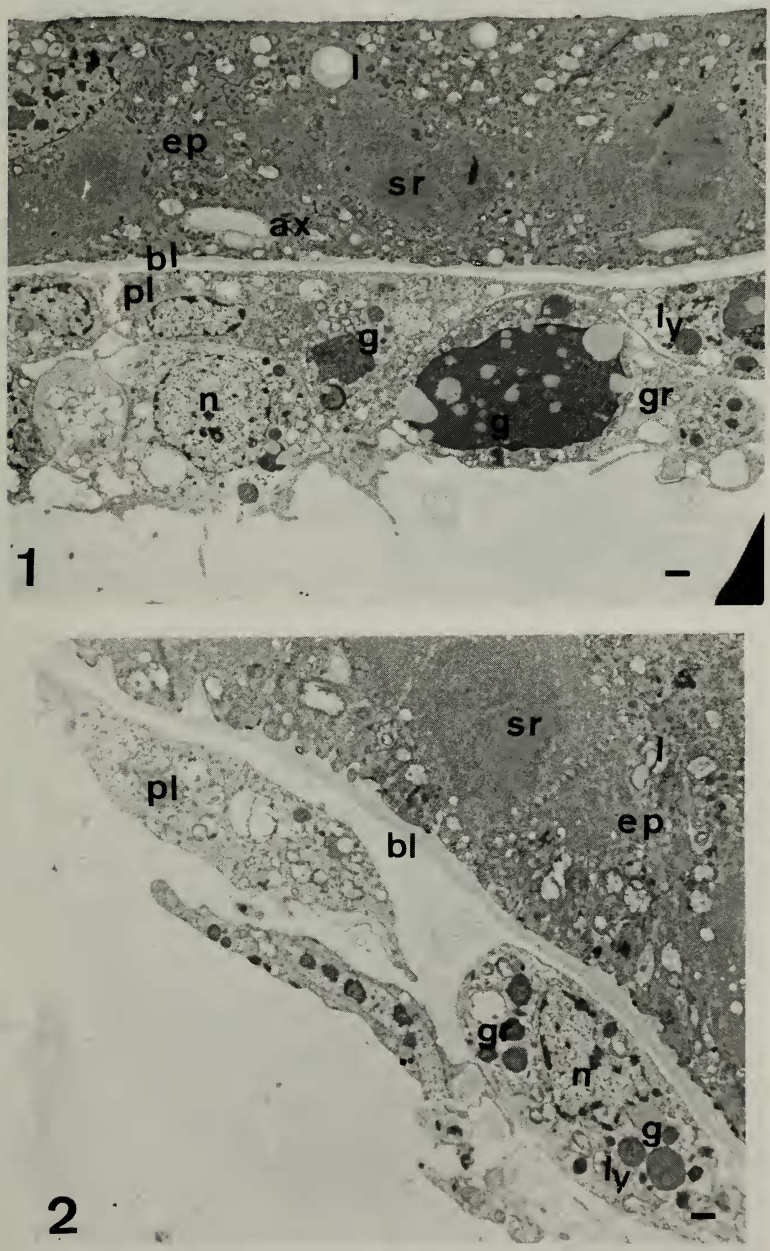
The specimens of *Apis dorsata* Fabricius, 1793, were collected in Bangalore, India, while visiting flowers, and therefore, were identified as forager workers. (This species is not found in Brazil neither as native or introduced species). The specimens were fixed in glutaraldehyde for electron microscopy; the dorsal tegument of abdomen of three different individuals was dissected into the component tergites. Thereupon they were refixed in 2,5% glutaraldehyde in 0,1M Na cacodylate buffer, pH 7.2 at 4°C for 2-4h, and post fixed in 1% osmium tetroxide in the same buffer. The tergites were individually embbeded in Epon-Araldite. Thin sections were stained with uranyl acetate and lead citrate as usual, then examined and photographed in a Zeiss EM9S2 transmission electron microscope, and the remaining emblocked material deposited in the Departamento de Biologia, Universidade Estadual Paulista, Rio Claro, SP.

RESULTS

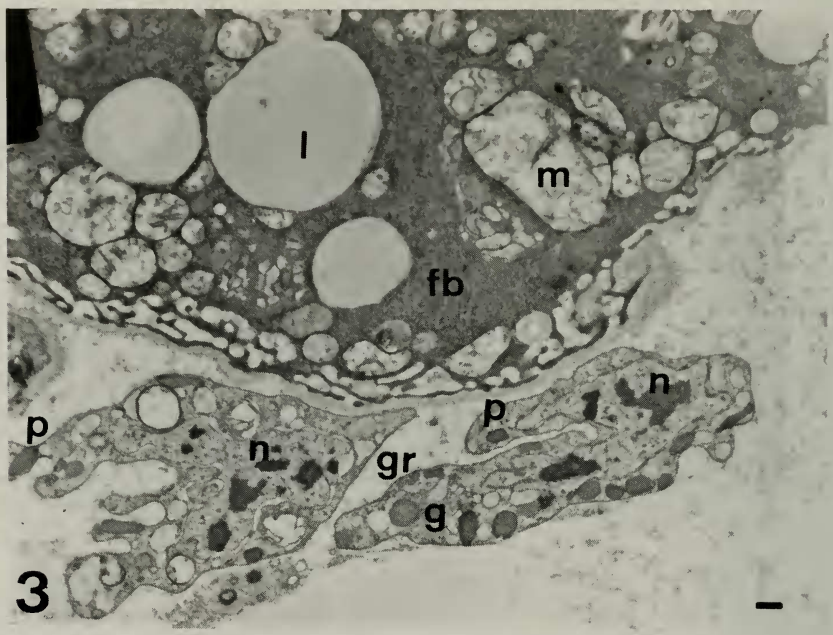
Two types of haemocytes were observed underlying to the tegument of III, IV and V tergites of the bee abdomen. In the IV segment the haemocytes are packed together and organized as a continuous layer underlying the epidermis (fig. 1), while in segments III and V, the cells are also adherent to the epidermis but the layer is discontinuous (figs. 2,3). The haemocytes attached to the epidermis have elongated or irregular forms (figs. 1-3), though some spherical cells were also observed, placed slightly away from the epithelium (fig. 4).

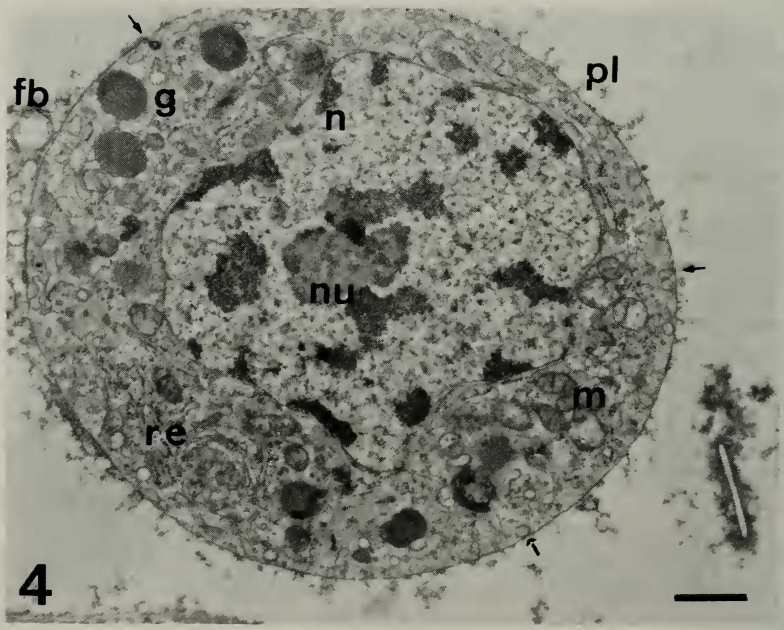
The two types of haemocytes were identified as plasmatocytes (PL) and granulocytes (GR). The PL appear as elongated or spherical cells, presenting few small granules, with electrondense, homogeneous content, and small, clear, vacuoles in the cytoplasm. The nucleus presents masses of heterochromatin lining internally the nuclear envelop and its shape follows that of the cell (figs. 1,2,4). The GRs, more frequent than PLs, are rather eliptic or irregular cells, the cytoplasm being filled with granules of various sizes, shapes and electron densities. Some of the haemocytes have pseudopodia-like projections of the cell surface, suggesting the possibility of locomotion and phagocytosis (fig. 1). However, phagocytic activity was not effectively observed, despite of the presence of lysosomes (fig. 1).

The epidermis of the adult bee, in non glandular regions, is formed by a very flat epithelium, without signs of cellular activity, but in the segments where the haemocytes were present the epidermal cells were enlarged and show signs of a post-secretory stage. The cells are probably involved with the lipid metabolism for they show masses of compacted smooth endoplasmic reticulum (fig. 2) and lipid droplets in the cytoplasm. This morphology is similar to that of a wax gland in post-secretory phase, undergoing



Figs. 1,2; *Apis dorsata*: 1, Continuous layer of granulocytes below the epidermis of the IV tergite; 2, discontinuous layer of haemocytes below the epidermis of III tergite. (ax, axon; bl, basal lamina; ep, epithelium; g, dense granules; gr, granulocytes; l, lipid; ly, lysosomes; n, nuclei; pl, plasmatocytes; sr, smooth endoplasmic reticulum).Bar = 1 μm.





Figs. 3,4. Haemocytes of *Apis dorsata*. 3, granulocytes(gr) showing pseudopodia-like projections(p); 4, round plasmatocyte (rl) showing pinocytic vesicles (arrows) at the surface. (fb, fat body; g, granules; l, lipid; m, mitochondria, n, nucleus; nu, nucleolus; pl, plasmatocytes; re, routh endoplasmic reticulum). Bar= 1 µm.

reabsorption process. Some cellular fragments, probably resulting from fat body cells desintegration, were observed close to the haemocytes (fig. 4).

DISCUSSION

Apis is a genus with few species of which only *Apis mellifera* L., 1758 has been well studied from the morphological and physiological respects. *Apis dorsata* is a related, more primitive species whose study is important to understanding some of the bee's traits, in the group evolution . The observed increase in height of the epidermis of the III, IV and V tergites in adult of *A.dorsata* was unexpected according to the *A.mellifera* pattern. Workers of *A.mellifera* do not have epithelial glands in the tergites, but only in the III to VI sternites. On turn the queens of these bees bear epithelial glands, that develop continuously throughout their lifetime, but are restricted to the III tergite (MOTA & CRUZ-LANDIM, 1988). There is no report of the presence of a glandular epithelium in the other tergites of any caste or life period of *A.mellifera,* however BILLEN & DUMORTIER (1986) reported the presence of tergal unicellular glands in workers of an orphan colony of *A.mellifera*, a gland type expected in queens, but this is a different kind of gland and an abnormal condition in the colony.

The haemocytes association to the epithelium has been observed in all specimens studied, restricted to the III, IV and V tergites. In all other tergites the epidermis was formed by very flat cells, as expected for non-glandular regions of the tegument, and there were no haemocytes attached to it. The formely mentioned morphological observations seem to indicate that the haemocytes, here described for *A.dorsata*, would be involved in the reabsorption processes of the overlying glandular epithelium. The exact mechanism of this involvement could not yet be ascertained, since the observations were only morphological ones. There are suggestions of a phagocytic process; but the epithelium remains intact despite signs of non-funcitonality. Some evidence for granules liberation from the GR have been observed by CROSSLEY (1979) and by the autor and Silva de Moraes. One possibility would be that chemical or enzymatic interactions might occur between the glandular epithelium and the underlying haemocytes.

REFERENCES

ASHURST, D.E. 1979. Hemocytes and connective tissue: a critical assessment. **In**: GUPTA, A.P. ed. **Insect Hemocytes: development, forms, functions and techniques**. New York, Cambridge Univ. p. 319-330.

BIDOCHKA, M.J. & KHACHATOURIAN, G.G. 1987. Hemocytic defense response to the entomopathogenic fungus *Beauveria bassiana* in the migratory grasshopper *Melanoplus sanguinipes*. **Entomol. exp. appl.** Dordrecht, **45**:151-156.

BILLEN, J.P.J. & DUMORTIER, K.T.M. 1986. Plasticity of honeybee castes. Occurrence of tergal glands in workers. **Naturwissenschaften**, Heidelberg, **73**:332-333.

CROSSLEY, A.C. 1979. Biochemical and ultrastructural aspects of synthesis, storage and secretion in hemocytes. **In**: GUPTA, A.P. ed. **Insect Hemocytes: development, forms, functions and techniques.** New York, Cambridge Univ. p. 423-473.

CRUZ-LANDIM, C. & S.-DA-CUNHA, M.A. 1971. Transformações pós-embrionárias em *Melipona*

quadrifasciata anthidioides Lep. V. Hemócitos das fases imaturas (Hymenoptera, Apidae). **Revta bras. Biol.**, Rio de Janeiro, **31**:471-483.

CRUZ-LANDIM, C. & MOTA, M.H.V.B. 1993. Differences between the female castes and males of *Scaptotrigona postica depilis* (Hymenoptera, Apidae, Meliponinae) in the occurrence and ultrastructure of tegumentary exocrine glands. **Naturalia**, São Paulo, **18**:173-187.

FEIR, D. 1979. Cellular and humoral responses to toxic substances. **In**: GUPTA A.P. ed. **Insect Hemocytes: development, forms, functions and techniques.** New York, Cambridge Univ. p. 415-421.

FISHER, R.C. 1970. Changes in the composition of host haemolymph after attack by an insect parasitoid. **Nature,** London, **5253**:191-192;

GILLIAM, M.A. & SHIMANUKI, H. 1971. Blood cells of the worker honeybee. **J. Apic. Res.,** London, **10**:79-85.

GUPTA, A.P. 1979. Hemocyte types: their structures, synonymies, interrelationships, and taxonomic significance. **In**: GUPTA A.P. ed. **Insect Hemocytes: development, forms, functions and techniques**. New York, Cambridge Univ. p.85-127.

LAI-FOOK, J. 1970. Haemocytes in the repair of wounds in insect (*Rhodnius prolixus*). **J. Morph.,** New York, **130**:297-314.

MOTA, M.H.V.B. & CRUZ-LANDIM, C. 1988. Ocorrência e morfometria de glândulas tegumentares abdominais em *Apis mellifera* L. (Hymenoptera, Apidae). **Revta bras. Zool.,** Curitiba, **5**:119-154.

RATCLIFF, N.A. & ROWLEY, A.F. 1979. Role of hemocytes in defense against biological agents. **In**: GUPTA, A.P. ed. **Insect Hemocytes: development, forms, functions and techniques**. New York, Cambridge Univ. 331-414.

WAGO, H. 1982. Involvement of microfilaments in filopodial function of phagocytic granular cells of the silkworm, *Bombyx mori*. **Developmental Comp. Immunol.,** Elmsford, **6**:655-664.

WHITTEN, J.M. 1969. Haemocyte activity in relation to epidermal cell growth, cuticle secretion, and cell death in a metamorphosing cyclorrhaphan pupa. **J. Insect. Physiol.,** Oxford, **15**:763-778.

Recebido em 29.12.1994; aceito em 3.10.1995